

University of Groningen

## Controlling competence in *Bacillus subtilis*

Hamoen, LW; Venema, G; Kuipers, OP

*Published in:*  
Microbiology-Sgm

*DOI:*  
[10.1099/mic.0.26003-0](https://doi.org/10.1099/mic.0.26003-0)

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2003

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Hamoen, LW., Venema, G., & Kuipers, OP. (2003). Controlling competence in *Bacillus subtilis*: shared use of regulators. *Microbiology-Sgm*, 149(1), 9-17. <https://doi.org/10.1099/mic.0.26003-0>

### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

## Review

Correspondence  
Oscar P. Kuipers  
o.p.kuipers@biol.rug.nl

# Controlling competence in *Bacillus subtilis*: shared use of regulators

Leendert W. Hamoen, Gerard Venema and Oscar P. Kuipers

Department of Genetics, Groningen Biomolecular Sciences and Biotechnology Institute,  
University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands

Bacteria have developed a wide arsenal of survival strategies to cope with the specific problems posed by their environment. These processes are carefully regulated and complex signal transduction cascades ensure proper activation of the adequate adaptive response. An intriguing observation is that generally the regulation pathways of the different adaptive processes are highly intertwined. In this review, this phenomenon is illustrated by the regulation of genetic competence development in *Bacillus subtilis*. The different regulation pathways which make up the gene regulation network that controls the development of competence are described, and their connections to other adaptive processes in *B. subtilis* are discussed.

## Overview

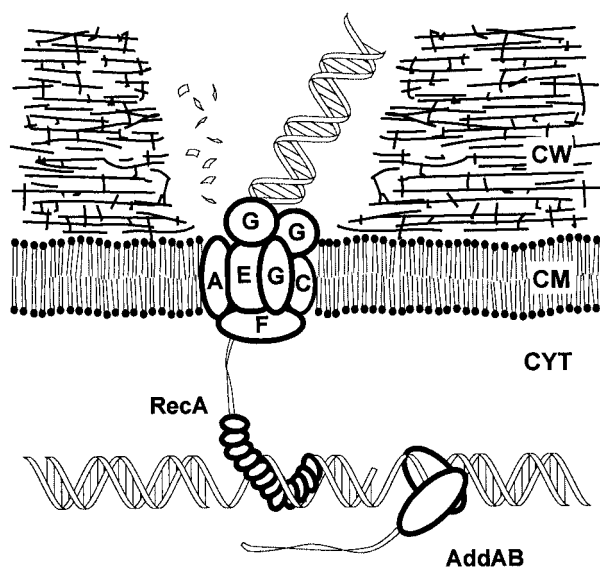
Bacteria are ubiquitous and extremely successful life forms. Their success is mainly a consequence of their talent of adapting to a large diversity of environments. To properly regulate the various adaptations, bacteria contain dedicated sensing devices linked to complex gene regulation systems. Due to the specific conditions to which these processes are tuned, it is reasonable to assume that each specific adaptive process contains its own specific set of sensing and regulatory proteins. However, it turns out that in many cases no unique, adaptive-process-specific gene regulation system exists. Instead, bacteria use highly intertwined networks of sensing and regulation pathways which conduct several different adaptive processes simultaneously. A striking example is the regulation of competence development in the Gram-positive bacterium *Bacillus subtilis*. In this paper, we review the regulation pathways involved in competence development, and describe how these pathways are linked to other adaptive processes in this organism.

*B. subtilis* is a soil-dwelling organism. In order to cope with the strong nutritional and physical fluctuations related to such an environment, this bacterium developed a wide arsenal of survival strategies. These processes can be conveniently observed in the laboratory by growing *B. subtilis* under batch-fermentation conditions. At the end of the exponential growth phase, when nutrients become limiting for optimal growth, *B. subtilis* cells start to synthesize a complex motility and chemotaxis system, which, in a natural habitat, would enable them to search for nutrients in the environment. If nutritional limitation continues, these motile cells enter the stationary growth phase, and start to secrete degradative enzymes such as proteases to liberate nutrients from alternative resources that are normally difficult to access. In addition, cells start to produce

antibiotics to fight off possible competitors. Prolonged nutritional stress results in the development of competence, and ultimately in sporulation of the bacterial population. Sporulation provides the bacterium with a way to survive extended harsh environmental conditions.

## Genetically competent *B. subtilis* cells

Genetic competence is a cellular differentiation process which converts *B. subtilis* cells into naturally transformable cells. In order to incorporate DNA from the medium, *B. subtilis* cells synthesize a specific DNA-binding and -uptake system, schematically presented in Fig. 1. Currently, five different loci have been identified which are involved in this DNA transport: *comC*, *comE*, *comF*, *comG* and *nucA*. Sequence comparisons revealed similarities between *comG* and type-IV pilins from *Pseudomonas* species and other pilin-like complexes. These similarities, together with experimental data, led to a model in which it is proposed that DNA uptake is accomplished by a pilin-like structure, composed of several *comG*-operon-encoded proteins (Chung *et al.*, 1998; reviewed by Dubnau, 1999). ComC appears to be involved in the correct assembly of this structure. The *comE* operon encodes a polytopic transmembrane protein (ComEC) which is thought to form a pore that guides the DNA into the cell interior, where it may associate with the DNA-helicase-resembling protein encoded by the *comF* operon (Londono-Vallejo & Dubnau, 1994; Provvedi & Dubnau, 1999). There is no nucleotide sequence specificity for DNA binding and DNA uptake, and competent *B. subtilis* cells can incorporate plasmid DNA, phage DNA or chromosomal DNA. DNA uptake is accompanied by endonucleolytic cleavage catalysed by the membrane localized nuclease NucA, and results in linear fragments up to 20 kb in size (Provvedi *et al.*, 2001). These fragments are taken up in single-stranded form. The complementary



**Fig. 1.** Schematic presentation of the DNA-uptake and DNA-integration process active in a competent *B. subtilis* cell (for details see main text). The number and configuration of the proteins which form the DNA translocation complex are drawn arbitrarily (A, NucA; C, ComC; E, ComE; F, ComF; G, ComG; CW, cell wall; CM, cell membrane; CYT, cytoplasm).

strands are degraded, and nucleotides are released into the medium. After internalization, the single-stranded DNA presumably associates with the general DNA-recombination protein RecA, and catalyses, together with other recombination proteins such as the DNA helicase AddAB, the integration of the newly acquired DNA into the genome (Haijema *et al.*, 1996).

The expression of the DNA-binding, -uptake and -recombination genes is controlled by the competence transcription factor ComK (van Sinderen *et al.*, 1995). The induction of this protein is strictly regulated. ComK expression occurs only when exponential growth ceases, and reaches its optimum after 2 h stationary growth. A sufficiently high cell density is a prerequisite for competence to develop optimally. Medium constituents are important regulatory factors as well, and the highest ComK expression is obtained in minimal medium with glucose as sole carbon source. An intriguing aspect of competence regulation in *B. subtilis* is that, even when all conditions appear to be optimal, only about 10–20 % of the cells in a culture will produce ComK and will develop competence (for a comprehensive review see Dubnau, 1993).

### Priming of *comK* expression

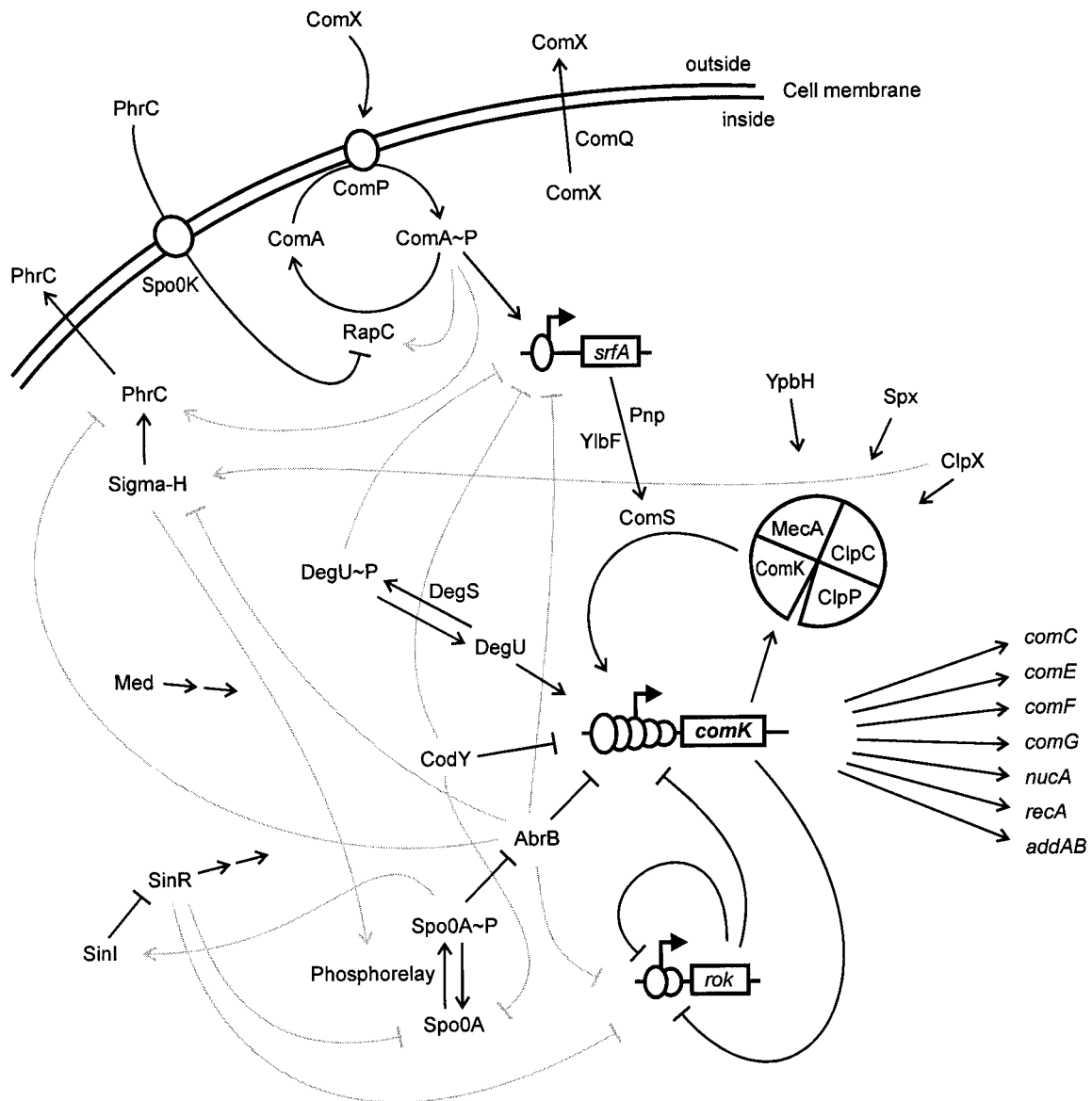
ComK binds to the *comK* promoter and induces its own transcription (van Sinderen & Venema, 1994). It is this auto-stimulatory loop which is a critical step in establishing competence (the regulation pathways described in this review are schematically visualized in Fig. 2). ComK binds

to various promoters as a tetramer composed of two dimers. In the *comK* promoter, the ComK-dimer binding sites are separated by 31 bp. This considerable distance hampers interaction between the dimers and the formation of a stable ComK tetramer. However, in between the ComK-dimer binding sites the transcription factor DegU can bind. It was shown that the presence of this protein strongly stimulates binding of ComK (Hamoen *et al.*, 2000). Since over-production of ComK bypasses the need for DegU, ComK appears to be able to function without DegU if present at sufficiently high concentrations (Roggiani & Dubnau, 1993). Apparently, the main function of DegU is to stimulate binding of ComK at the onset of competence development, when the number of ComK molecules is insufficient for binding to the *comK* promoter. Thus expression of *comK* requires DegU to 'prime' the auto-stimulatory *comK* transcription loop.

During the transition from exponential to stationary growth, *B. subtilis* starts to secrete a large number of degradative enzymes. The two-component regulatory system DegS/DegU plays an important role in the regulation of this process (Kunst *et al.*, 1988). Several mutations in either the protein kinase DegS or the DegU response regulator provoke hyper-production of degradative enzymes, presumably as a result of increased DegU phosphorylation (Dahl *et al.*, 1992; Henner *et al.*, 1988). These so-called 'Hy-phenotypes' are very pleiotropic, resulting in decreased competence, diminished motility and glucose-insensitive sporulation (Henner *et al.*, 1988). The unphosphorylated form of DegU binds efficiently to the *comK* promoter, and several genetic experiments led to the conclusion that unphosphorylated DegU is required for competence, whereas DegU~P is essential for the production of degradative enzymes. Thus the DegS/DegU two-component system provides *B. subtilis* with a developmental switch between different adaptive processes (Msadek *et al.*, 1995). The nature of the signal that activates DegS is unknown.

### Transcriptional repression of *comK* expression

When cells become competent, DNA replication is blocked and cell division ceases (Haijema *et al.*, 2001). The competence transcription factor ComK appears to be responsible for these phenomena, so accurate control of *comK* expression is vital to *B. subtilis* (Hahn *et al.*, 1995b). Due to the auto-stimulatory transcription of *comK*, premature expression of this gene must somehow be prevented. In the early days of sporulation research, a mutation was identified, *spo0A*, which, in addition to inhibiting sporulation and production of several proteases, also affected competence. The protein encoded by *spo0A* exhibits all the characteristics of a response regulator (Green *et al.*, 1991). However, Spo0A is not activated by a single sensor protein, like most of the two-component signal transduction systems, but is phosphorylated via a complex phosphorelay system, comprising several histidine kinases (KinA, B and C), an intermediate phosphoacceptor (Spo0F), and a phosphotransferase (Spo0B) (Burbulys *et al.*, 1991). The phosphorelay somehow



**Fig. 2.** Overview of the different regulation pathways involved in competence development (for details see main text). The main pathways are indicated by solid black lines. The grey lines illustrate a number of regulatory connections described in the text. The *srfA*, *comK* and *rok* genes are schematically depicted with the promoter regions to which several transcription factors are bound (circles). The ComP and Spo0K proteins are depicted in the cell membrane. The ComK/MecA/CipC/CipP protein complex is encircled. Arrows and T-bars indicate positive and negative regulation, respectively.

It is a small DNA-binding protein and acts as a general transcriptional repressor of various genes involved in stationary phase processes such as sporulation (e.g. *spoVG*), degradative enzyme production (e.g. *aprE*), amino acid utilization (e.g. *dpp*) and antibiotic production (e.g. *tycA*) (Ferrari *et al.*, 1988; Robertson *et al.*, 1989; Slack *et al.*, 1991). During growth, expression of *abrB* declines as a result of transcriptional repression by activated Spo0A (O'Reilly & Devine, 1997). Disruption of *spo0A* results in overproduction of ABrB, as a consequence of which expression of many stationary-phase genes is inhibited, including expression of

*comK* (Hahn *et al.*, 1995a). Recently, it was found that AbrB binds to the *comK* promoter and occupies the transcription initiation region (L. Hamoen, unpublished data). Although an important function of AbrB in the competence development pathway is to prevent the premature expression of *comK*, it also has a stimulatory effect on competence development, as judged from the observation that mutations in *abrB* decrease competence (Hahn *et al.*, 1995a). Why this is so is not yet known.

AbrB is not the only regulator which inhibits transcription of the *comK* promoter. Expression of *comK* is sensitive to the amino acid composition of the medium. Amino-acid-mediated repression of several *B. subtilis* genes has been shown to depend on a GTP-binding transcription factor, CodY (Slack *et al.*, 1995). CodY senses the intracellular GTP concentration as an indication of the nutritional conditions in the medium, and regulates many stationary growth phase genes such as the dipeptide transport operon *dpp*, but also for example *spo0A* (Ratnayake-Lecamwasam *et al.*, 2001). A *codY* mutation alleviated the amino-acid-imposed control of *comK* expression, and DNA-footprinting analyses revealed that CodY occupies the RNA polymerase binding site of the *comK* promoter as well (Serror & Sonenshein, 1996).

In an extensive genetic analysis, Hoa *et al.* (2002) identified a third repressor of the *comK* promoter. Overexpression of the gene *ykuW* inhibited transcription of *comK*, and a knockout of *ykuW* resulted in ComK overproduction. *ykuW* was renamed *rok*, an acronym for 'repressor of *comK*'. Rok binds and represses not only the *comK* promoter but also its own promoter. High levels of ComK repress *rok* expression as well, and it was shown that ComK binds specifically to the promoter of *rok*. The ComK-dependent inhibition of *rok* expression appears to transform the Rok control of *comK* transcription into a positive feedback loop. Presumably, Rok fulfils a more pleiotropic role in *B. subtilis* as changes in expression levels of this protein not only influence *comK* expression but also affect sporulation. By now a total of five different transcription factors have been identified which control the *comK* promoter, a rather remarkable number for a prokaryotic promoter.

### Cell-density-dependent induction of competence

Bacteria are able to communicate with each other through the production of 'quorum-sensing' pheromones. Accumulation of pheromones in the growth medium signals the presence of a sufficient number of congeners (a quorum), and triggers various cell-density-dependent processes. In *B. subtilis*, two competence-stimulating pheromones have been identified. The main competence-stimulating factor, ComX, is a 9 to 10 amino acid oligopeptide with an isoprenyl modification of the tryptophan residue (Ansaldi *et al.*, 2002; Magnuson *et al.*, 1994). *comQ*, the gene located upstream of *comX*, is required for the post-translational modifications and secretion of the ComX pheromone. Downstream of *comX* are located the genes of a two-component regulatory

system, *comA* and *comP* (Weinrauch *et al.*, 1990). The membrane-spanning protein kinase ComP senses the accumulation of ComX in the medium, and responds by phosphorylating ComA (Solomon *et al.*, 1995). The transcription factor ComA is required for the expression of *srfA*, a locus which was shown to be essential for competence development (Hahn & Dubnau, 1991; Nakano *et al.*, 1991; van Sinderen *et al.*, 1990). Sequencing of the entire *srfA* locus revealed a huge 30 kb spanning operon, encoding a very large protein complex responsible for the non-ribosomal synthesis of the lipopeptide antibiotic surfactin (Cosmina *et al.*, 1993). It was shown that phosphorylation of ComA stimulates the binding of ComA to the *srfA* promoter, and as such induces *srfA* transcription and surfactin production (Roggiani & Dubnau, 1993). Surprisingly, the synthesis of this antibiotic had nothing to do with competence development. Located within the second open reading frame of the *srfA* operon is a small gene which encodes a 46-amino-acid peptide. It was this small peptide, designated ComS, that turned out to be essential for competence to develop (D'Souza *et al.*, 1994; Hamoen *et al.*, 1995). By embedding *comS* within *srfA*, *B. subtilis* elegantly uses a single quorum-sensing pathway for two different adaptive processes. Production of surfactin, which is a potent biosurfactant, may not only give *B. subtilis* cells a selective advantage by eliminating competitors, but may also allow the bacterium to use the lytic action of surfactin, so that potentially valuable genetic material released by lysed micro-organisms can be incorporated.

The competence-stimulating factor (CSF) was the second competence pheromone identified. Amino acid sequence analysis indicated that this pheromone is a pentapeptide with an amino acid sequence similar to the C-terminal part of a 40 amino acid long secreted protein, PhrC (Solomon *et al.*, 1996). How processing of PhrC to CSF occurs is unknown (for clarity we will use here the nomenclature PhrC instead of CSF). Also PhrC exerts its quorum-sensing activity at the level of *srfA* transcription, and requires ComP and ComA for its activity. However, the stimulatory effect of PhrC on *srfA* expression also requires the oligopeptide permease Spo0K, suggesting that sensing of this pheromone occurs intracellularly (Lazazzera *et al.*, 1997). Upstream of *phrC*, the gene *rapC* is located. Genetic studies indicated that RapC is a negative regulator of *srfA* expression and probably dephosphorylates ComA ~ P (Solomon *et al.*, 1996). On the basis of a homologous system involved in sporulation (RapA/PhrA), it is supposed that PhrC inhibits RapC activity (*phr* stands for phosphatase regulator). Thus when PhrC levels rise sufficiently, RapC activity is repressed, ComA ~ P levels accumulate, and *srfA/comS* expression increases (Solomon *et al.*, 1996). So both pheromones, ComX and PhrC, stimulate expression of *srfA/comS*. Why *B. subtilis* uses a double quorum-sensing pathway for this process is not clear.

The concentration-dependent effectiveness of antibiotics explains why surfactin production depends on the accumulation of congeners. Yet quorum sensing may also have an important function in the genetic transformation process.

Due to a RecA-based homologous recombination system active during competence, the efficiency of recombination and integration with the *B. subtilis* genome largely depends upon the measure of DNA sequence homology. Consequently, competent *B. subtilis* cells are transformed most efficiently with DNA from congeners. It is as if competence has primarily evolved to exchange genetic material within the species. This is even more apparent for *Haemophilus influenzae* and *Neisseria gonorrhoeae* (Lorenz & Wackernagel, 1994). In these bacteria, DNA binding and DNA uptake depend on specific sequence motifs dispersed over the genome as a result of which only DNA from the same or closely related species can be taken up. For *Streptococcus pneumoniae* it was shown that the development of competence is accompanied by lysis and DNA release of a subfraction of the population, and both processes appeared to be stimulated by the same quorum-sensing regulation pathway (Steinmoen *et al.*, 2002). Therefore, natural competence can be considered as the bacterial attempt at a sexual life-cycle (Redfield, 1988). As the preferred donor DNA presumably originates from dead and lysed congeners, the cell density dependence of competence development may therefore have become an evolutionarily valuable asset.

### Post-translational control

Mutations in two loci, *mecA* and *mecB*, resulted in overproduction of ComK (Kong *et al.*, 1993). *mecB* was later renamed *clpC*, since the protein encoded by this gene resembled the heat-shock protein ClpC of *Escherichia coli* (Msadek *et al.*, 1994). The *mec*-phenotype suggested that MecA and ClpC are negative regulators of competence. It required purification of these proteins to reveal their precise function. Biochemical analyses indicated that MecA interacts with ComK, and ClpC interacts with MecA (Turgay *et al.*, 1997). ClpC alone does not bind ComK, but in the presence of MecA a stable ternary complex is established. ClpC is a member of the ubiquitous HSP100 chaperone-like family, and several members of this family form a complex with the conserved protease ClpP. *In vitro* experiments indicated that *B. subtilis* ClpC and ClpP interact with each other as well (Turgay *et al.*, 1998). Moreover, the addition of purified ClpP to the ternary ComK/MecA/ClpC complex resulted in rapid degradation of ComK. Thus MecA recruits ComK to the ClpCP protease complex for degradation, and in this way prevents auto-transcriptional activation of *comK*.

Expression of neither *mecA* nor *clpC* changes dramatically during the transition to the stationary growth phase. The question of how the MecA/ClpC-mediated inactivation of ComK is alleviated remained unanswered, until it was observed that purified ComS inhibited the *in vitro* formation of a stable ternary ComK/MecA/ClpC complex (Turgay *et al.*, 1997). It was later shown that ComS binds to MecA and stimulates the degradation of both proteins by the ClpCP protease complex (Ogura *et al.*, 1999; Persuh *et al.*, 1999). In conclusion, synthesis of ComS protects ComK from degradation so that auto-stimulation of *comK* expression is initiated. Thus the competence pheromones

ComX and PhrC, which stimulate *comS* transcription, ultimately control *comK* expression on the post-translational level, by means of regulated proteolysis of ComK.

### More research, more regulators

As the research of competence development proceeds, the list of regulators that are involved in competence is growing. For example, there is MedA, a membrane protein which decreases expression of *comK* (Ogura *et al.*, 1997). The function of MedA is unclear; a deletion of *medA* reduces *comK* expression considerably, yet the expression of *comG* is unaffected, and cells become competent.

A regulator which does influence competence development is SinR. The *sin* locus encodes another regulatory couple of which the activity extends beyond a single adaptive process. The first protein encoded by this bicistronic operon, SinI, represses the activity of the transcriptional regulator SinR, encoded by the second gene (Gaur *et al.*, 1988). A *sinR* disruption results in non-motile, filamentous cells, and a strong reduction in competence, whereas overproduction of SinR blocks sporulation and related protease production. The regulation of SinR is complex, and control at both the transcriptional and (post)translational level seems to occur (Smith, 1993). Transition from exponential to stationary growth is accompanied by an increase in SinI production. SinI shows similarity with the C-terminal part of SinR, and inactivates SinR by forming a heteromultimer with this protein (Bai *et al.*, 1993). The position of SinR in the competence signal transduction cascade is somewhat controversial. Liu *et al.* (1996) suggested that SinR is required for optimal production of ComS, whereas Hahn *et al.* (1996) presented evidence suggesting that SinR is directly involved in *comK* transcription. Recently, more evidence for the latter proposition has been found. It appeared that SinR acts negatively on *rok* transcription, and that inactivation of *rok* could bypass the requirement of SinR for *comK* expression (Hoa *et al.*, 2002).

One of the latest additions to the list of known competence regulators is YlbF (Tortosa *et al.*, 2000). A disruption in *ylbF* affects both competence and sporulation. Although the exact function of YlbF is unknown, genetic studies suggest that this protein is required for the production of sufficient amounts of ComS. Actually, the production of ComS is rather complex since it also requires polynucleotide phosphorylase (Pnp) (Luttinger *et al.*, 1996). Pnp is involved in RNA stability, and besides stimulating the synthesis of surfactin synthetase and ComS, this RNA-binding protein is also required for adaptation to growth at low temperatures.

Controlled proteolysis plays an essential role in the regulation of many cellular developmental processes, and it is therefore not surprising that most of the newly discovered components in the competence signal transduction network are somehow involved in this process. Mutations affecting the activity of the proteases concerned show very pleiotropic phenotypes. For example, in *B. subtilis* blocking the

expression of the protease ClpP disturbs competence development, motility, degradative enzyme production and sporulation (Msadek *et al.*, 1998). The absence of ClpP results in high levels of MecA. Since binding of MecA to ComK reduces the activity of ComK, high MecA levels will prevent development of competence, despite the absence of ClpP-dependent proteolyses of ComK. ClpP can also form an ATP-dependent protease complex with the chaperone ClpX, and a disruption of *clpX* interferes with the development of competence as well. In such a mutant the expression of *comS* appears to be insufficient to prevent the MecA-mediated degradation of ComK. A disruption in a gene called *spx* suppresses the adverse effects of *clpP* or *clpX* mutations (Nakano *et al.*, 2002). Protein-binding studies indicated that Spx forms a complex with MecA and ClpCP, and enhances the binding of ComK to this protein complex. As a result, higher concentrations of ComS are required to release ComK from the complex and prevent the proteolytic degradation of ComK. The role of Spx in the competence signal transduction cascade is unclear. A disruption of *spx* has no consequence for the development of competence, and expression of *spx* is very low under wild-type conditions. It is only when ClpP or ClpX is absent that Spx reaches levels high enough to obstruct the activity of ComS.

Due to the general use of ATP-dependent proteases in so many cellular processes, substrate interference can be an important factor in gene regulation. An example which illustrates this phenomenon is the MecA paralogue YpbH. When the concentration of YpbH is disturbed in the cell, the expression of *comK* changes, since YpbH binds to ClpC as well (Persuh *et al.*, 2002).

### Further intertwinement of the regulation circuitry

An important regulatory mechanism in bacterial differentiation which has not been addressed so far is the use of different sigma factors to control transcription. *B. subtilis* has specific sigma factors for expression of the motility and chemotaxis apparatus (sigma-D), for the induction of the general stress response (sigma-B), and most strikingly, for sporulation, in which a succession of several sigma factors (sigma-E, F, G and K) directs the development into the mother cell and forespore (Haldenwang, 1995). In the development of competence, sigma factors play a minor role, as most proteins concerned use the major (house-keeping) sigma factor sigma-A. However, one additional sigma factor is known to be required for competence, the minor sigma factor sigma-H, encoded by *spo0H*. Expression of sigma-H is low, but the level increases at the end of exponential growth. Various sigma-H-controlled genes have been identified, including the phosphorelay components: *kinA*, *spo0F* and *spo0A* (Predich *et al.*, 1992; Siranosian & Grossman, 1994). *phrC* contains a sigma-H promoter as well, and the major role of sigma-H in competence development appears to be the production of the quorum-sensing pheromone PhrC (Solomon *et al.*, 1996).

The main pathways of the competence signal transduction cascade are depicted in Fig. 2 with black lines. However, this scheme remains a simplification, as the different regulatory pathways are not separate entities but are mutually intertwined. A few examples have already been mentioned, such as sigma-H, which is required for the expression of phosphorelay components and PhrC. Yet many more cross-links in the regulation circuitry of Fig. 2 can be drawn (grey lines). For instance, ClpX influences sigma-H activity (Liu *et al.*, 1999), high concentrations of phosphorylated DegU reduce *srfA* transcription (Hahn & Dubnau, 1991), SinR is a repressor of the *spo0A* promoter (Mandic-Mulec *et al.*, 1995), and also represses *rok* expression (Hoa *et al.*, 2002), and CodY exerts its repressive action on both *comK* and *srfA* transcription (Serror & Sonenshein, 1996). AbrB negatively controls, apart from *comK* transcription (Hahn *et al.*, 1995a), the production of PhrC (Solomon *et al.*, 1995), and expression of *spo0H* (Weir *et al.*, 1991) and *rok* (Hoa *et al.*, 2002), whereas Spo0A represses *abrB*, but stimulates expression of *sinI* (Gaur *et al.*, 1988). Finally, the quorum-sensing pathway gets increasingly complicated in view of the observation that several *rap/phr* operons, including *rapA/phrA* and *rapC/phrC*, are also regulated by the ComP/ComA two-component system (Lazazzera *et al.*, 1999; Perego *et al.*, 1996).

### Conclusions

*comK* expression depends on the presence of SinR, DegU, ComS and a minimal amount of AbrB. However, sufficient levels of these proteins are only transiently present during growth. ComS appears when the cell density increases, levels of AbrB decrease during growth, DegU becomes phosphorylated, and SinR is ultimately inactivated by SinI. Apparently, only a restricted time window exists in which conditions are optimal for *comK* expression, which may explain why only a small percentage of cells in a *B. subtilis* culture become competent. Whether there is any evolutionary advantage for this numerical limitation remains speculation. From the point of view of the species, it is not necessarily an advantage to have every cell competent, as long as a sufficient number of cells is transformable and able to enrich their genetic material. In addition, it seems as if the development of competence excludes the production of degradative enzymes, since SinR represses degradative enzyme production, but is required for competence development, and phosphorylation of DegU is required for the production of degradative enzymes, but not for competence development. Possibly, the physiological status of competent cells does not support efficient expression and secretion of degradative enzymes. In this scenario, the small number of competent cells will take advantage of the degradative enzymes secreted by the majority of non-competent cells. Since this will require a reasonable concentration of degradative enzymes in the medium, this may also explain the cell-density-dependence of competence development.

It is remarkable that, for a radical differentiation process like competence, *B. subtilis* uses almost no regulatory proteins

that are exclusively used in this process. For several years the ComS/MecA/ComK regulation pathway was considered to be solely involved in competence regulation. However, it was shown later that a *mecA*-null mutation inhibits expression of sigma-D, the sigma factor involved in motility and chemotaxis, and that this inhibition partially depended on ComK (Liu & Zuber, 1998; Rashid *et al.*, 1996). Competence is not unusual in having almost no unique 'private' regulators at its disposal. In fact, the various stationary-phase processes in *B. subtilis* share the majority of their regulators. A strong interlock between gene regulatory pathways appears to be a common fact among prokaryotes. Looking at the regulation of general adaptive processes in bacteria, such as catabolite repression, and heat- and cold-shock response, it is apparent that the regulation cascades are composed of many general pleiotropic regulatory proteins. So is there an evolutionary advantage of interlocking gene regulatory pathways? 'Efficiency' might be the key to this question. Because various transcriptional regulators, depending on where they bind a promoter, can function positively as well as negatively, the use of shared regulators is an efficient mechanism for coordinating and discriminating between different cellular responses. It links regulatory pathways and as such ensures a tight coordination between them. In addition, cells need lesser regulatory proteins as a result of which they handle their metabolic resources more economically.

The phenomenon of interlocked gene regulatory circuits has also been related to stability and robustness of the system (Little *et al.*, 1999). A stable network will maintain its state in the face of small perturbations (stochastic fluctuations) of input signals and network components. It has been suggested, and recently experimentally supported, that especially negative feedback loops provide such stability (Becskei & Serrano, 2000). Negative feedback loops are commonly present in complex gene regulatory circuits. In this review, several examples have been described, yet, in case of competence development it is questionable whether stability is an important reason for the intertwinement of the gene regulatory pathways. The experience with competence development in *B. subtilis* teaches that the fraction of cells which ultimately become competent fluctuates considerably, despite use of carefully controlled growth conditions.

Robustness of gene regulatory circuits refers to the sensitivity of systems towards changes in the biochemical parameters of the components brought about by genetic alterations of the components (Little *et al.*, 1999). Particularly in organisms with an active and promiscuous genetic exchange system, such as genetic competence in *B. subtilis*, it can be envisioned that robustness of gene regulatory circuits is vital.

The full measure of complexity of gene regulation networks complicates the ascertainment of properties such as stability and robustness. The rise of whole-genome transcription measurements with DNA arrays, and developments in high-throughput two-hybrid analyses, makes it possible to study these phenomena at a new level. Several *in silico* modelling

studies with extensive gene regulation networks, based on transcriptome and proteome data from yeast, provided evidence that 'connectivity' increases the stability and robustness of gene regulation networks (Featherstone & Broadie, 2002; Maslov & Sneppen, 2002; Wagner, 2000). In the near future, such *in silico* studies will be achievable with *B. subtilis* and other prokaryotes. By then it will be interesting to examine whether the competence signal transduction network is more stable than gene regulation networks of bacteria which live in stable environments, or more robust than gene regulation networks of bacteria which do not become genetically competent.

## Acknowledgements

This work was supported by the Netherlands Organization of Scientific Research (NWO) under the auspices of the Netherlands Foundation for Chemical Research (SON), and by the Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen.

## References

- Ansaldo, M., Marolt, D., Stebe, T., Mandic-Mulec, I. & Dubnau, D. (2002). Specific activation of the *Bacillus* quorum-sensing systems by isoprenylated pheromone variants. *Mol Microbiol* **44**, 1561–1573.
- Bai, U., Mandic-Mulec, I. & Smith, I. (1993). SinI modulates the activity of SinR, a developmental switch protein of *Bacillus subtilis*, by protein-protein interaction. *Genes Dev* **7**, 139–148.
- Becskei, A. & Serrano, L. (2000). Engineering stability in gene networks by autoregulation. *Nature* **405**, 590–593.
- Bird, T. H., Grimsley, J. K., Hoch, J. A. & Spiegelman, G. B. (1993). Phosphorylation of Spo0A activates its stimulation of *in vitro* transcription from the *Bacillus subtilis* *spoIIG* operon. *Mol Microbiol* **9**, 741–749.
- Burbulys, D., Trach, K. A. & Hoch, J. A. (1991). Initiation of sporulation in *B. subtilis* is controlled by a multicomponent phosphorelay. *Cell* **64**, 545–552.
- Chung, Y. S., Breidt, F. & Dubnau, D. (1998). Cell surface localization and processing of the ComG proteins, required for DNA binding during transformation of *Bacillus subtilis*. *Mol Microbiol* **29**, 905–913.
- Cosmina, P., Rodriguez, F., de Ferra, F., Grandi, G., Perego, M., Venema, G. & van Sinderen, D. (1993). Sequence and analysis of the genetic locus responsible for surfactin synthesis in *Bacillus subtilis*. *Mol Microbiol* **8**, 821–831.
- Dahl, M. K., Msadek, T., Kunst, F. & Rapoport, G. (1992). The phosphorylation state of the DegU response regulator acts as a molecular switch allowing either degradative enzyme synthesis or expression of genetic competence in *Bacillus subtilis*. *J Biol Chem* **267**, 14509–14514.
- D'Souza, C., Nakano, M. M. & Zuber, P. (1994). Identification of *comS*, a gene of the *srfA* operon that regulates the establishment of genetic competence in *Bacillus subtilis*. *Proc Natl Acad Sci U S A* **91**, 9397–9401.
- Dubnau, D. (1993). Genetic exchange and homologous recombination. In *Bacillus subtilis and Other Gram-positive Bacteria*, pp. 555–584. Edited by A. L. Sonenshein, J. A. Hoch & R. Losick. Washington, DC: American Society for Microbiology.



- Dubnau, D. (1999). DNA uptake in bacteria. *Annu Rev Microbiol* 53, 217–244.
- Featherstone, D. E. & Broadie, K. (2002). Wrestling with pleiotropy: genomic and topological analysis of the yeast gene expression network. *Bioessays* 24, 267–274.
- Ferrari, E., Henner, D. J., Perego, M. & Hoch, J. A. (1988). Transcription of *Bacillus subtilis* subtilisin and expression of subtilisin in sporulation mutants. *J Bacteriol* 170, 289–295.
- Gaur, N. K., Cabane, K. & Smith, I. (1988). Structure and expression of the *Bacillus subtilis* *sin* operon. *J Bacteriol* 170, 1046–1053.
- Green, B. D., Olmedo, G. & Youngman, P. (1991). A genetic analysis of Spo0A structure and function. *Res Microbiol* 142, 825–830.
- Hahn, J. & Dubnau, D. (1991). Growth stage signal transduction and the requirements for *srfA* induction in development of competence. *J Bacteriol* 173, 7275–7282.
- Hahn, J., Roggiani, M. & Dubnau, D. (1995a). The major role of Spo0A in genetic competence is to downregulate *abrB*, an essential competence gene. *J Bacteriol* 177, 3601–3605.
- Hahn, J., Bylund, J., Haines, M., Higgins, M. & Dubnau, D. (1995b). Inactivation of *mecA* prevents recovery from the competent state and interferes with cell division and the partitioning of nucleoids in *Bacillus subtilis*. *Mol Microbiol* 18, 755–767.
- Hahn, J., Luttinger, A. & Dubnau, D. (1996). Regulatory inputs for the synthesis of ComK, the competence transcription factor of *Bacillus subtilis*. *Mol Microbiol* 21, 763–775.
- Haijema, B. J., van Sinderen, D., Winterling, K., Kooistra, J., Venema, G. & Hamoen, L. W. (1996). Regulated expression of the *dinR* and *recA* genes during competence development and SOS induction in *Bacillus subtilis*. *Mol Microbiol* 22, 75–85.
- Haijema, B. J., Hahn, J., Haynes, J. & Dubnau, D. (2001). A ComGA-dependent checkpoint limits growth during the escape from competence. *Mol Microbiol* 40, 52–64.
- Haldenwang, W. G. (1995). The sigma factors of *Bacillus subtilis*. *Microbiol Rev* 59, 1–30.
- Hamoen, L. W., Eshuis, H., Jongbloed, J., Venema, G. & van Sinderen, D. (1995). A small gene, designated *comS*, located within the coding region of the fourth amino acid-activation domain of *srfA*, is required for competence development in *Bacillus subtilis*. *Mol Microbiol* 15, 55–63.
- Hamoen, L. W., Van Werkhoven, A. F., Venema, G. & Dubnau, D. (2000). The pleiotropic response regulator DegU functions as a priming protein in competence development in *Bacillus subtilis*. *Proc Natl Acad Sci U S A* 97, 9246–9251.
- Henner, D. J., Yang, M. & Ferrari, E. (1988). Localization of *Bacillus subtilis* *sacU*(Hy) mutations to two linked genes with similarities to the conserved procaryotic family of two-component signalling systems. *J Bacteriol* 170, 5102–5109.
- Hoa, T. T., Tortosa, P., Albano, M. & Dubnau, D. (2002). Rok (YkuW) regulates genetic competence in *Bacillus subtilis* by directly repressing *comK*. *Mol Microbiol* 43, 15–26.
- Kong, L., Siranosian, K. J., Grossman, A. D. & Dubnau, D. (1993). Sequence and properties of *mecA*, a negative regulator of genetic competence in *Bacillus subtilis*. *Mol Microbiol* 9, 365–373.
- Kunst, F., Debarbouille, M., Msadek, T., Young, M., Mauel, C., Karamata, D., Klier, A., Rapoport, G. & Dedonder, R. (1988). Deduced polypeptides encoded by the *Bacillus subtilis* *sacU* locus share homology with two-component sensor-regulator systems. *J Bacteriol* 170, 5093–5101.
- Lazazzera, B. A., Solomon, J. M. & Grossman, A. D. (1997). An exported peptide functions intracellularly to contribute to cell density signaling in *B. subtilis*. *Cell* 89, 917–925.
- Lazazzera, B. A., Kurtser, I. G., McQuade, R. S. & Grossman, A. D. (1999). An autoregulatory circuit affecting peptide signaling in *Bacillus subtilis*. *J Bacteriol* 181, 5193–5200.
- Little, J. W., Shepley, D. P. & Wert, D. W. (1999). Robustness of a gene regulatory circuit. *EMBO J* 18, 4299–4307.
- Liu, J. & Zuber, P. (1998). A molecular switch controlling competence and motility: competence regulatory factors ComS, MecA, and ComK control sigmaD-dependent gene expression in *Bacillus subtilis*. *J Bacteriol* 180, 4243–4251.
- Liu, J., Cosby, W. M. & Zuber, P. (1999). Role of lon and ClpX in the post-translational regulation of a sigma subunit of RNA polymerase required for cellular differentiation in *Bacillus subtilis*. *Mol Microbiol* 33, 415–428.
- Liu, L., Nakano, M. M., Lee, O. H. & Zuber, P. (1996). Plasmid-amplified *comS* enhances genetic competence and suppresses *sinR* in *Bacillus subtilis*. *J Bacteriol* 178, 5144–5152.
- Londono-Vallejo, J. A. & Dubnau, D. (1994). Mutation of the putative nucleotide binding site of the *Bacillus subtilis* membrane protein ComFA abolishes the uptake of DNA during transformation. *J Bacteriol* 176, 4642–4645.
- Lorenz, M. G. & Wackernagel, W. (1994). Bacterial gene transfer by natural genetic transformation in the environment. *Microbiol Rev* 58, 563–602.
- Luttinger, A., Hahn, J. & Dubnau, D. (1996). Polynucleotide phosphorylase is necessary for competence development in *Bacillus subtilis*. *Mol Microbiol* 19, 343–356.
- Magnuson, R., Solomon, J. & Grossman, A. D. (1994). Biochemical and genetic characterization of a competence pheromone from *B. subtilis*. *Cell* 77, 207–216.
- Mandic-Mulec, I., Doukhan, L. & Smith, I. (1995). The *Bacillus subtilis* SinR protein is a repressor of the key sporulation gene *spo0A*. *J Bacteriol* 177, 4619–4627.
- Maslov, S. & Sneppen, K. (2002). Specificity and stability in topology of protein networks. *Science* 296, 910–913.
- Msadek, T., Kunst, F. & Rapoport, G. (1994). MecB of *Bacillus subtilis*, a member of the ClpC ATPase family, is a pleiotropic regulator controlling competence gene expression and growth at high temperature. *Proc Natl Acad Sci U S A* 91, 5788–5792.
- Msadek, T., Kunst, F. & Rapoport, G. (1995). A signal transduction network in *Bacillus subtilis* includes the DegS/DegU and ComP/ComA two-component systems. In *Two-Component Signal Transduction*, pp. 447–471. Edited by J. A. Hoch & T. J. Silhavy. Washington, DC: American Society for Microbiology.
- Msadek, T., Dartois, V., Kunst, F., Herbaud, M. L., Denizot, F. & Rapoport, G. (1998). ClpP of *Bacillus subtilis* is required for competence development, motility, degradative enzyme synthesis, growth at high temperature and sporulation. *Mol Microbiol* 27, 899–914.
- Nakano, M. M., Magnuson, R., Myers, A., Curry, J., Grossman, A. D. & Zuber, P. (1991). *srfA* is an operon required for surfactin production, competence development, and efficient sporulation in *Bacillus subtilis*. *J Bacteriol* 173, 1770–1778.
- Nakano, M. M., Nakano, S. & Zuber, P. (2002). Spx (YjbD), a negative effector of competence in *Bacillus subtilis*, enhances ClpC-MecA-ComK interaction. *Mol Microbiol* 44, 1341–1349.
- Ogura, M., Ohshiro, Y., Hirao, S. & Tanaka, T. (1997). A new *Bacillus subtilis* gene, *med*, encodes a positive regulator of *comK*. *J Bacteriol* 179, 6244–6253.
- Ogura, M., Liu, L., Lacelle, M., Nakano, M. M. & Zuber, P. (1999). Mutational analysis of ComS: evidence for the interaction of ComS and MecA in the regulation of competence development in *Bacillus subtilis*. *Mol Microbiol* 32, 799–812.

- O'Reilly, M. & Devine, K. M. (1997). Expression of AbrB, a transition state regulator from *Bacillus subtilis*, is growth phase dependent in a manner resembling that of Fis, the nucleoid binding protein from *Escherichia coli*. *J Bacteriol* **179**, 522–529.
- Perego, M. (1998). Kinase-phosphatase competition regulates *Bacillus subtilis* development. *Trends Microbiol* **6**, 366–370.
- Perego, M., Glaser, P. & Hoch, J. A. (1996). Aspartyl-phosphate phosphatases deactivate the response regulator components of the sporulation signal transduction system in *Bacillus subtilis*. *Mol Microbiol* **19**, 1151–1157.
- Persuh, M., Turgay, K., Mandic-Mulec, I. & Dubnau, D. (1999). The N- and C-terminal domains of MecA recognize different partners in the competence molecular switch. *Mol Microbiol* **33**, 886–894.
- Persuh, M., Mandic-Mulec, I. & Dubnau, D. (2002). A MecA paralog, YpbH, binds ClpC, affecting both competence and sporulation. *J Bacteriol* **184**, 2310–2313.
- Predich, M., Nair, G. & Smith, I. (1992). *Bacillus subtilis* early sporulation genes *kinA*, *spo0F*, and *spo0A* are transcribed by the RNA polymerase containing sigma H. *J Bacteriol* **174**, 2771–2778.
- Provvedi, R. & Dubnau, D. (1999). ComEA is a DNA receptor for transformation of competent *Bacillus subtilis*. *Mol Microbiol* **31**, 271–280.
- Provvedi, R., Chen, I. & Dubnau, D. (2001). NucA is required for DNA cleavage during transformation of *Bacillus subtilis*. *Mol Microbiol* **40**, 634–644.
- Rashid, M. H., Tamakoshi, A. & Sekiguchi, J. (1996). Effects of *mecA* and *mecB* (*clpC*) mutations on expression of *sigD*, which encodes an alternative sigma factor, and autolysin operons and on flagellin synthesis in *Bacillus subtilis*. *J Bacteriol* **178**, 4861–4869.
- Ratnayake-Lecamwasam, M., Serror, P., Wong, K. W. & Sonenshein, A. L. (2001). *Bacillus subtilis* CodY represses early-stationary-phase genes by sensing GTP levels. *Genes Dev* **15**, 1093–1103.
- Redfield, R. J. (1988). Evolution of bacterial transformation: is sex with dead cells ever better than no sex at all? *Genetics* **119**, 213–221.
- Robertson, J. B., Gocht, M., Marahiel, M. A. & Zuber, P. (1989). AbrB, a regulator of gene expression in *Bacillus*, interacts with the transcription initiation regions of a sporulation gene and an antibiotic biosynthesis gene. *Proc Natl Acad Sci U S A* **86**, 8457–8461.
- Roggiani, M. & Dubnau, D. (1993). ComA, a phosphorylated response regulator protein of *Bacillus subtilis*, binds to the promoter region of *srfA*. *J Bacteriol* **175**, 3182–3187.
- Serror, P. & Sonenshein, A. L. (1996). CodY is required for nutritional repression of *Bacillus subtilis* genetic competence. *J Bacteriol* **178**, 5910–5915.
- Siranosian, K. J. & Grossman, A. D. (1994). Activation of *spo0A* transcription by sigma H is necessary for sporulation but not for competence in *Bacillus subtilis*. *J Bacteriol* **176**, 3812–3815.
- Slack, F. J., Mueller, J. P., Strauch, M. A., Mathiopoulos, C. & Sonenshein, A. L. (1991). Transcriptional regulation of a *Bacillus subtilis* dipeptide transport operon. *Mol Microbiol* **5**, 1915–1925.
- Slack, F. J., Serror, P., Joyce, E. & Sonenshein, A. L. (1995). A gene required for nutritional repression of the *Bacillus subtilis* dipeptide permease operon. *Mol Microbiol* **15**, 689–702.
- Smith, I. (1993). Regulatory proteins that control late-growth development. In *Bacillus subtilis and Other Gram-positive Bacteria*, pp. 555–584. Edited by A. L. Sonenshein, J. A. Hoch & R. Losick. Washington, DC: American Society for Microbiology.
- Solomon, J. M., Magnuson, R., Srivastava, A. & Grossman, A. D. (1995). Convergent sensing pathways mediate response to two extracellular competence factors in *Bacillus subtilis*. *Genes Dev* **9**, 547–558.
- Solomon, J. M., Lazazzera, B. A. & Grossman, A. D. (1996). Purification and characterization of an extracellular peptide factor that affects two different developmental pathways in *Bacillus subtilis*. *Genes Dev* **10**, 2014–2024.
- Steinmoen, H., Knutsen, E. & Havarstein, L. S. (2002). Induction of natural competence in *Streptococcus pneumoniae* triggers lysis and DNA release from a subfraction of the cell population. *Proc Natl Acad Sci U S A* **99**, 7681–7686.
- Strauch, M. A., Spiegelman, G. B., Perego, M., Johnson, W. C., Burbulys, D. & Hoch, J. A. (1989). The transition state transcription regulator AbrB of *Bacillus subtilis* is a DNA binding protein. *EMBO J* **8**, 1615–1621.
- Strauch, M., Webb, V., Spiegelman, G. & Hoch, J. A. (1990). The SpoOA protein of *Bacillus subtilis* is a repressor of the *abrB* gene. *Proc Natl Acad Sci U S A* **87**, 1801–1805.
- Tortosa, P., Albano, M. & Dubnau, D. (2000). Characterization of *ylbF*, a new gene involved in competence development and sporulation in *Bacillus subtilis*. *Mol Microbiol* **35**, 1110–1119.
- Turgay, K., Hamoen, L. W., Venema, G. & Dubnau, D. (1997). Biochemical characterization of a molecular switch involving the heat shock protein ClpC, which controls the activity of ComK, the competence transcription factor of *Bacillus subtilis*. *Genes Dev* **11**, 119–128.
- Turgay, K., Hahn, J., Burghoorn, J. & Dubnau, D. (1998). Competence in *Bacillus subtilis* is controlled by regulated proteolysis of a transcription factor. *EMBO J* **17**, 6730–6738.
- van Sinderen, D. & Venema, G. (1994). *comK* acts as an autoregulatory control switch in the signal transduction route to competence in *Bacillus subtilis*. *J Bacteriol* **176**, 5762–5770.
- van Sinderen, D., Withoff, S., Boels, H. & Venema, G. (1990). Isolation and characterization of *comL*, a transcription unit involved in competence development of *Bacillus subtilis*. *Mol Gen Genet* **224**, 396–404.
- van Sinderen, D., Luttinger, A., Kong, L., Dubnau, D., Venema, G. & Hamoen, L. (1995). *comK* encodes the competence transcription factor, the key regulatory protein for competence development in *Bacillus subtilis*. *Mol Microbiol* **15**, 455–462.
- Wagner, A. (2000). Robustness against mutations in genetic networks of yeast. *Nat Genet* **24**, 355–361.
- Weinrauch, Y., Penchev, R., Dubnau, E., Smith, I. & Dubnau, D. (1990). A *Bacillus subtilis* regulatory gene product for genetic competence and sporulation resembles sensor protein members of the bacterial two-component signal-transduction systems. *Genes Dev* **4**, 860–872.
- Weir, J., Predich, M., Dubnau, E., Nair, G. & Smith, I. (1991). Regulation of *spo0H*, a gene coding for the *Bacillus subtilis* sigma H factor. *J Bacteriol* **173**, 521–529.